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STUDIES OF THE KINETICS OF POTASSIUM EXCHANGE BETWEEN CELLS  
AND PLASMA OF CANINE BLOOD IN VITRO USING K<sup>42</sup>

by

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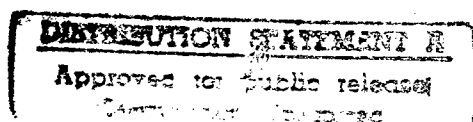
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ABSTRACT

Freshly drawn heparinized canine whole blood was equilibrated in vitro (paraffin lined vessels) at  $38.1^{\circ}\text{C}$  with a normal pulmonary atmosphere saturated with water vapor. It was then tagged by mixing with a small amount of plasma containing dextrose and radioactive  $\text{KCl}$ . The potassium content of the resulting plasma was thus raised by not more than 3% of the normal and the blood sugar to about 400 mg%. By this procedure cells were maintained in a healthy state for periods up to 10 hours as shown by minimal hemolysis, nearly constant hematocrit and minimal potassium leakage.

In whole blood the activity of the plasma decreased initially at a rapid rate which was correlated with the appearance of activity in the cells (red cells, white cells, and platelets). The initial decrease followed an exponential curve to an elevated base line, half of this change being completed in about 50 minutes. However, if the white cells and platelets were previously removed by repeated differential centrifugation the behavior was different. The plasma activity declined very slowly, at the rate of about 1% per hour.

The initial rapid decrease in plasma activity for whole blood was attributed to a rapid exchange of potassium in the fraction containing the white cells and platelets. A typical value for the amount of this easily exchangeable potassium is about 30% of the total potassium in the plasma. It is evident that studies of potassium exchange of erythrocytes must give equivocal results unless the white cells and platelets are removed in advance or otherwise taken into account.

\* \* \* \* \*

The rate at which potassium exchanges between the plasma and erythrocytes of normal blood remains a matter of considerable physiological interest. It is well known that the rate of exchange may be determined by the use of the radioactive isotope  $K^{42}$ . In Table 1 we present a summary of previous investigations of this type including only the results of studies of potassium exchange in blood.

In all of this work it has been assumed that the exchange of potassium between cells and plasma represents erythrocyte exchange and that exchange between the plasma and the fraction containing the white cells and platelets is unimportant. We have recently observed potassium exchange in vitro in heparinized whole dog blood for periods averaging about 10 hours. Here the disappearance of radioactive potassium from the plasma and its appearance in the cellular fraction was different from that which occurs in samples of blood from which all platelets and white cells have been removed.

Table 1.

Reference	Date	Species	Results and comments
Hahn, Hevesy and Rebbe <sup>1</sup>	1939	Rabbit	The specific activity of the cells reaches only 3% of that of the plasma in 24 hours in vitro and in vivo. Only 4 single determinations are presented.
Eisenman, Ott, Smith, and Winkler <sup>2</sup>	1940	Human	No detectable exchange is found in 4 hours in vitro but the experimental uncertainty is as much as 8%.
Cohn <sup>3</sup>	1940	Human, rat, dog,	Some evidence is obtained of initial rapid exchange, the rate later becoming slow.
Noonan, Fenn, and Haeger <sup>4</sup>	1941	Rat	The exchange in vivo is initially rapid, the rate later decreasing so that the specific activity of the cells relative to the plasma increases by 5% per hour
Dean, Noonan, Haeger, and Fenn <sup>5</sup>	1941	Rabbit, rat	The specific activity of the cells reaches 29% of that of the plasma in 6 hours in the rabbit, 55% in 10 hours in the rat, both studies being in vivo. The in vitro experiments using cells of rabbit, man, and rat in Ringer's solution are controversial.
Mullins, Fenn Noonan, and Haeger <sup>6</sup>	1941	Man, dog, rat, cat, guinea pig, frog.	The time for the specific activity of the cells to reach 30% of that of the plasma ranges from 0.8 hours in the cat to 16 hours in the frog. Much of the data shows extreme experimental uncertainty, compensated in part by a large number of determinations.

## METHODS

Radioactive potassium ( $K^{42}$ ) was prepared by exposing  $K_2CO_3$  to slow neutrons in the Oak Ridge chain reactor. This material was an aliquot of the lot used in the isotope distribution program and contained considerably less than 0.05% of sodium. The active material was converted to isotonic KCl by a remote titration process to be described in a later report. Blood was obtained by jugular puncture using a few drops of heparin as an anticoagulant. The donors were healthy mongrel dogs.

Two types of in vitro techniques will be described. In the first approximately 10 cc of blood was taken under mineral oil and delivered under oil into a polystyrene tube kept at 38°C in a water bath. It was then tagged with 10 cubic millimeters of 1.2%  $K^{42}Cl$  and carefully mixed with a glass plunger coated with paraffin. Samples of 1.5 cc were then taken by pipette and quickly delivered into small glass centrifuge tubes with ground stoppers (Figure 1). The tubes were coated on the inside with paraffin and the stoppers were quickly sealed in by briefly warming in a small flame and then cooling under running water. Although the volume of gas in the tubes was small we attempted in some cases to minimize the loss of  $CO_2$  from the blood to this volume by breathing alveolar air into the tubes before sealing. As each tube was filled and sealed it was placed in the water bath and turned end for end at 1 revolution per minute. At selected times tubes were removed from the bath and centrifuged. The plasma was removed and the cells were washed twice with cold isotonic saline or sucrose solution. The radioactivity of plasma, wash solution, and cells was then determined.

This technique was found unsatisfactory for accurate work for the following reasons:

- (a) The method of control of  $\text{CO}_2$  leakage from the blood is questionable.
- (b) Gradual hemolysis of the blood developed so that after about 5 hours definite traces could be seen in the plasma by careful inspection.
- (c) Temperature control was poor since samples were distributed into the tubes at room temperature while the exchange of  $\text{K}^{42}$  was in progress.
- (d) Quick and clean separation of cells from plasma could not be affected and it was shown that the washing procedure produced some potassium leakage. This is in accord with the findings of others.<sup>7</sup> The time of equilibration was the interval from the instant of tagging to the time of first washing, and was in error by the small amount of exchange occurring during the washing process.

In spite of these limitations smooth curves were usually obtained. Measurements were made both of the activity of the plasma and of the cells. Figure 2 shows a typical result.

The second method which we will now describe provided observations only of samples of plasma and whole blood, but avoided many of the objections which apply to the first procedure. The heparinized blood was pipetted into paraffin lined flasks (Figure 3). These flasks contain 2 compartments. In one we placed 3 cc of blood and in the other 1/2 cc of plasma containing added dextrose and radioactive  $\text{KCl}$ . The quantity of dextrose was sufficient to raise the blood sugar of the final mixture of blood and plasma to about 400 mg%. The total added potassium increased the plasma potassium content of the final mixture by not more than 3% of the normal value. The flasks were capped and connected to a gas circulating system by which the atmosphere was controlled during the experiment. The gas was kept saturated with water vapor and contained sufficient  $\text{CO}_2$  to maintain the buffer system of the plasma intact. After equilibration in the water bath at  $38.1^\circ\text{C}$  for about 1/2 hour the flasks were rocked at 10 oscillations per minute. Since the contents of both compartments were quickly mixed, the exchange of  $\text{K}^{42}$  began at this point. The plane which included the axis of each cell and its partition made an angle of 45 degrees with the axis of oscillation so that the blood poured over alternate ends of the partition at each oscillation which insured good agitation with a minimum of mechanical injury. The paraffin coating prevented smearing of the blood over the vessel walls.

At various intervals the flasks were removed. Samples were then taken for hematocrit determinations, for whole blood analyses, and for analyses of plasma. The plasma was separated in a typical clinical centrifuge, the time of separation being taken as one minute after starting the motor. Hematocrit determinations were done by the high speed air turbine method described by Parpart and Ballentine.<sup>8</sup> Whole blood and plasma samples were analyzed as follows. One cc was diluted to 25 cc in a volumetric flask. A 5-cc sample was evaporated in a porcelain ashing capsule and its radioactivity determined on a typical GM counter of the bell type. The remainder

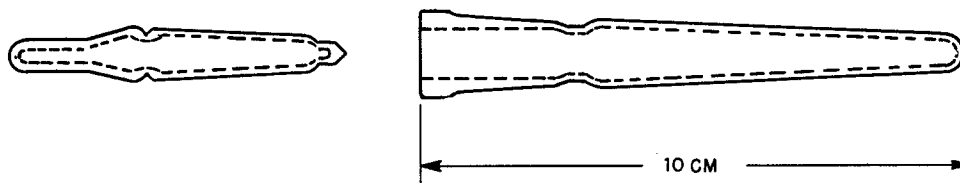


Figure 1. Tube used in preliminary equilibration experiments.

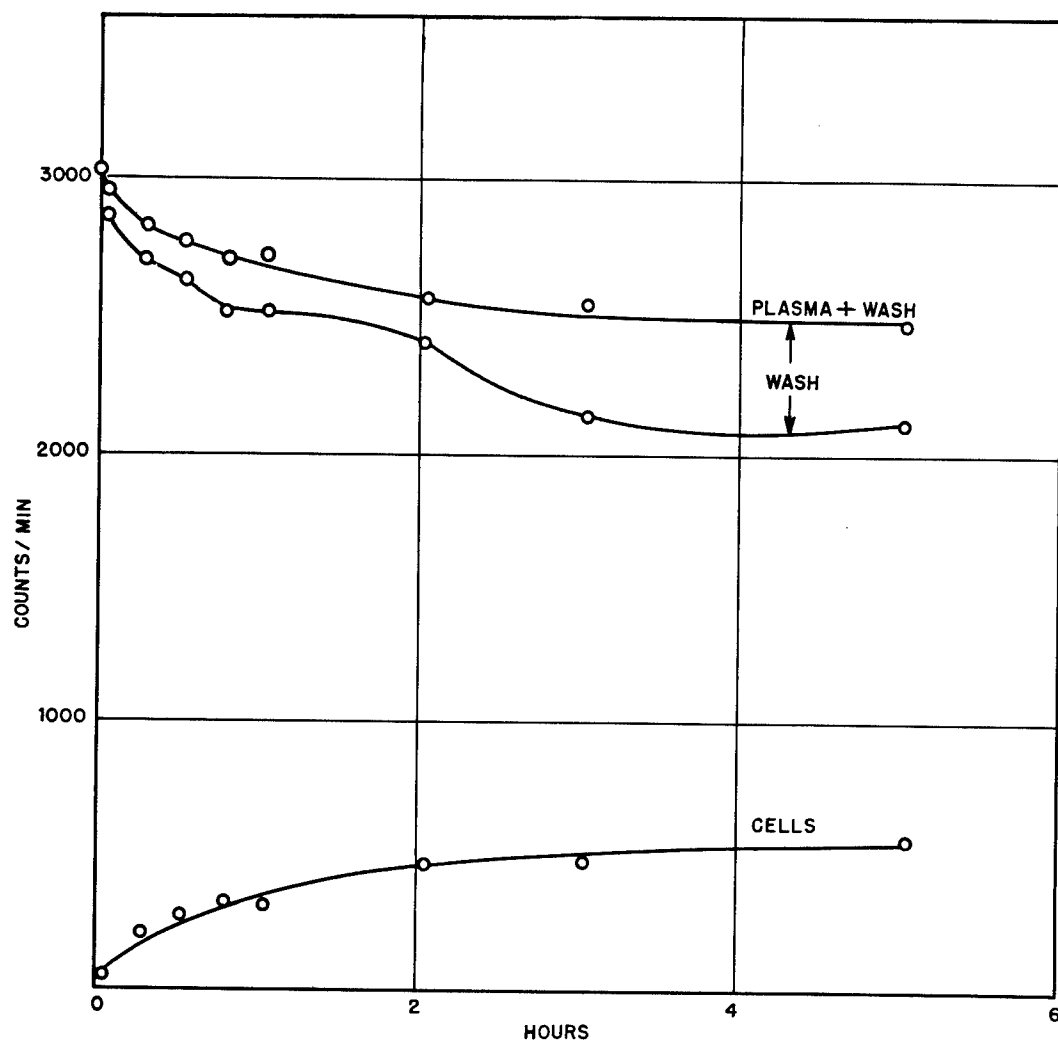


Figure 2. A typical preliminary exchange experiment showing the radioactivity of plasma, cells, and plasma plus wash solution as ordinates; time in hours as abscissas.

was allowed to decay to safe levels and was analyzed for total potassium by a Perkin-Elmer Model 52A Flame Photometer. A careful record was kept of evidence of hemolysis, which was observed by visual inspection of the plasma after centrifugation. In general this was found to be below the limit of visual detection during most of the course of an experiment but the last samples usually showed very slight but detectable traces of hemoglobin.

## RESULTS

Figure 4 shows one of our better exchange curves for whole blood equilibrated with an approximately venous atmosphere. Arterialized blood showed essentially the same behavior. It may be seen that the initial decrease in plasma activity was rapid but that this was superimposed upon an

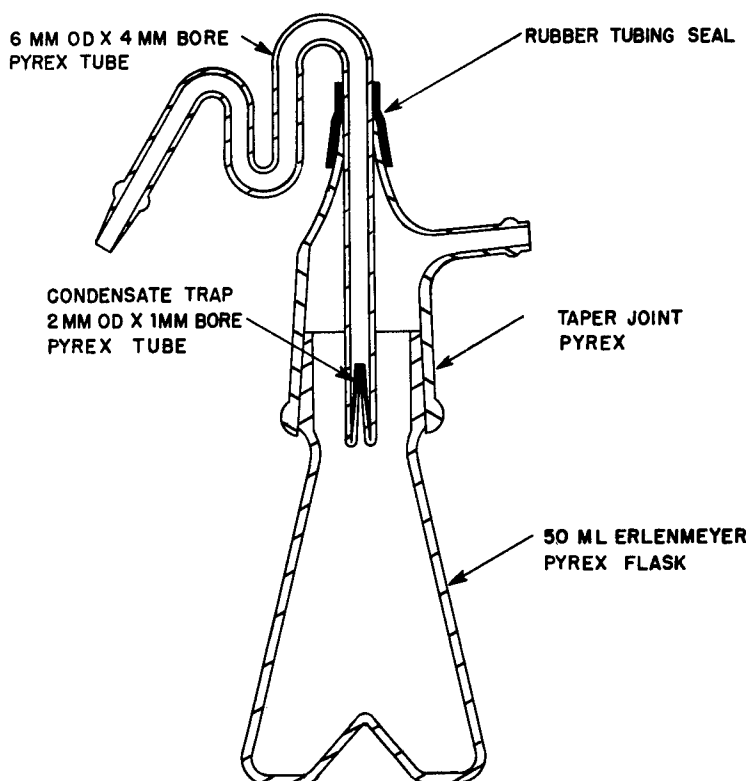


Figure 3. Equilibration flask used in later experiments showing central partition and the connections for gas circulation.

elevated base line which changed very slowly. The hematocrit record and potassium content of the plasma is shown in Table 2. It may be seen that the experimental conditions were at first quite good although some deterioration probably occurred as time progressed. The results are in fair agreement with the curves already presented which were obtained by the earlier method if we admit some loss due to washing in the earlier experiments. We arbitrarily selected the activity of the plasma at 5 hours as a base line and subtracted this value from those at earlier times. A semi-logarithmic plot of the results is shown in Figure 5. It may be seen that this portion of the exchange process conforms fairly well to the familiar exponential pattern. The change in plasma radioactivity approached  $1/2$  the base line value in about 50 minutes. We realize that our base line selection here was somewhat arbitrary and the value for the half value time is uncertain as a result.

After establishing the exchange behavior in whole blood we proceeded to remove the white cells and platelets. This was done by centrifuging carefully, and gently removing the top layer of cells, this procedure being repeated 3 times until white cells could rarely be found in the blood by direct smear. After some practice this could be done without producing more than a slight hemolysis which was detectable only by close inspection against a white background. A typical curve obtained with this blood is shown in Figure 6. The hematocrit record and changes in plasma potassium concentration present a picture similar to that in the previous experiment (Table 2).

Table 2. Typical record of hematocrits and plasma potassium concentrations of samples at the time of analysis.

Sample number	Time after tagging (minutes)	Hematocrit (percent)	Concentration of potassium in plasma (mg. percent) *
0	2	40.3	17.8
1	17	40.3	18.2
2	35	40.3	18.2
3	49	39.9	18.2
4	66	40.5	18.5
5	90	39.9	19.2
6	124	41.1	20.0
7	188	41.0	19.5
8	304	41.0	22.5 †
9	420	40.7	21.6 †
10	540	41.6	20.9

\* The values are uncorrected for viscosity error and sodium interference in the flame photometer.

† The sample had wet the wall of the flask during the experiment.

#### NORMAL CONDITION OF CELLS

Strictly speaking, the cellular elements of blood cannot be said to be entirely normal after removal from the donor, and this is one criticism of any in vitro experiment. In general the burden of proof, that the phenomena which are under observation are those which occur in the circulation, is on the observer who conducts such experiments. Here we were dealing with cells which were suspended in a plasma containing an anticoagulant. The buffer system had been disturbed but later reestablished. Any waste products of metabolism were allowed to accumulate and the source of energy (dextrose) was added artificially. Nevertheless, an inspection of the results indicates that the influence of these factors on the cells was much less in these experiments than in some others found in the earlier literature. We cite a few examples.

Although Mullins and his associates state that no difference could be found in results using various anticoagulants, Ponder and his associates have shown<sup>9</sup> that the use of an abnormal anti-coagulant, such as oxalate, has a profound effect at least on the exchangeability of water in certain mammalian erythrocytes. We, therefore, feel that caution is warranted in the choice of an anti-coagulant in the present work. Here the best choice is probably heparin, a substance actually found in the circulation.

We have relied on the normal buffer system of the plasma to maintain the proper pH. Although this has been temporarily disturbed, the pH of typical samples maintained under our experimental conditions has been measured and found within normal limits after equilibration. It seems

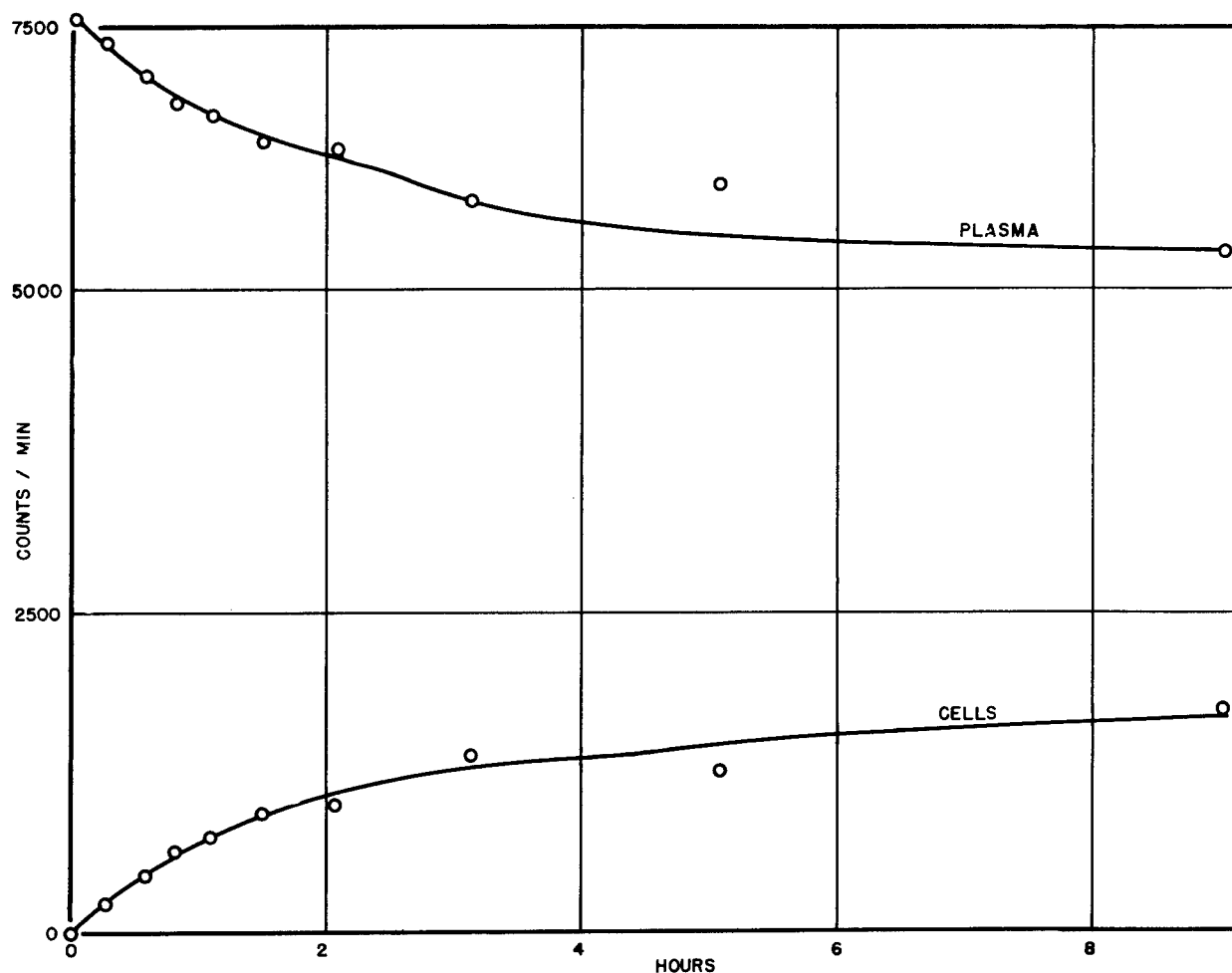


Figure 4. A typical curve for whole blood obtained by the improved technique showing activities as ordinates and equilibration times in hours as abscissas. The upper curve represents the plasma activities, the lower curve the activities of the cells obtained by difference.

surprising in the work previously cited that pure nitrogen or carbon monoxide could have a negligible effect on the results of in vitro experiments of this sort.

The added potassium disturbs the normal electrolyte content of the plasma minimally. This is in contrast to the work of Eisenman, et al, who added large amounts of potassium usually elevating the plasma level by a factor of 10 or more.

We observed in several preliminary experiments that when no sugar was added the cells tended to deteriorate within 5 or 6 hours after removal from the donor. The blood sugar was found to disappear quite rapidly. This fact is of course a familiar one and has been recently reviewed by Peters and Van Slyke.<sup>10</sup> The amount of sugar added raises the level of reducing sugars in the plasma to about 4 times normal. It greatly extends the time before severe hemolysis sets in. Although above normal, the level thus obtained is still well within the range of that found in a diabetic animal.



## CONCLUSION

The exchange of radioactive potassium in those erythrocytes which remained after removal of the fraction containing white cells and platelets evidently proceeded at the slow but detectable rate of about 1% per hour. This exchange was much slower than that observed in vitro with canine blood by Mullins and his associates. Allowing for the rapidly exchanging fraction, which probably was included in their experiments on whole blood as in ours, the difference was not reconciled. The information which they present as to their in vitro methods is insufficient for us to investigate this discrepancy further. Their in vivo experiments show four points. Three are in fair agreement with our findings and one is in complete disagreement.

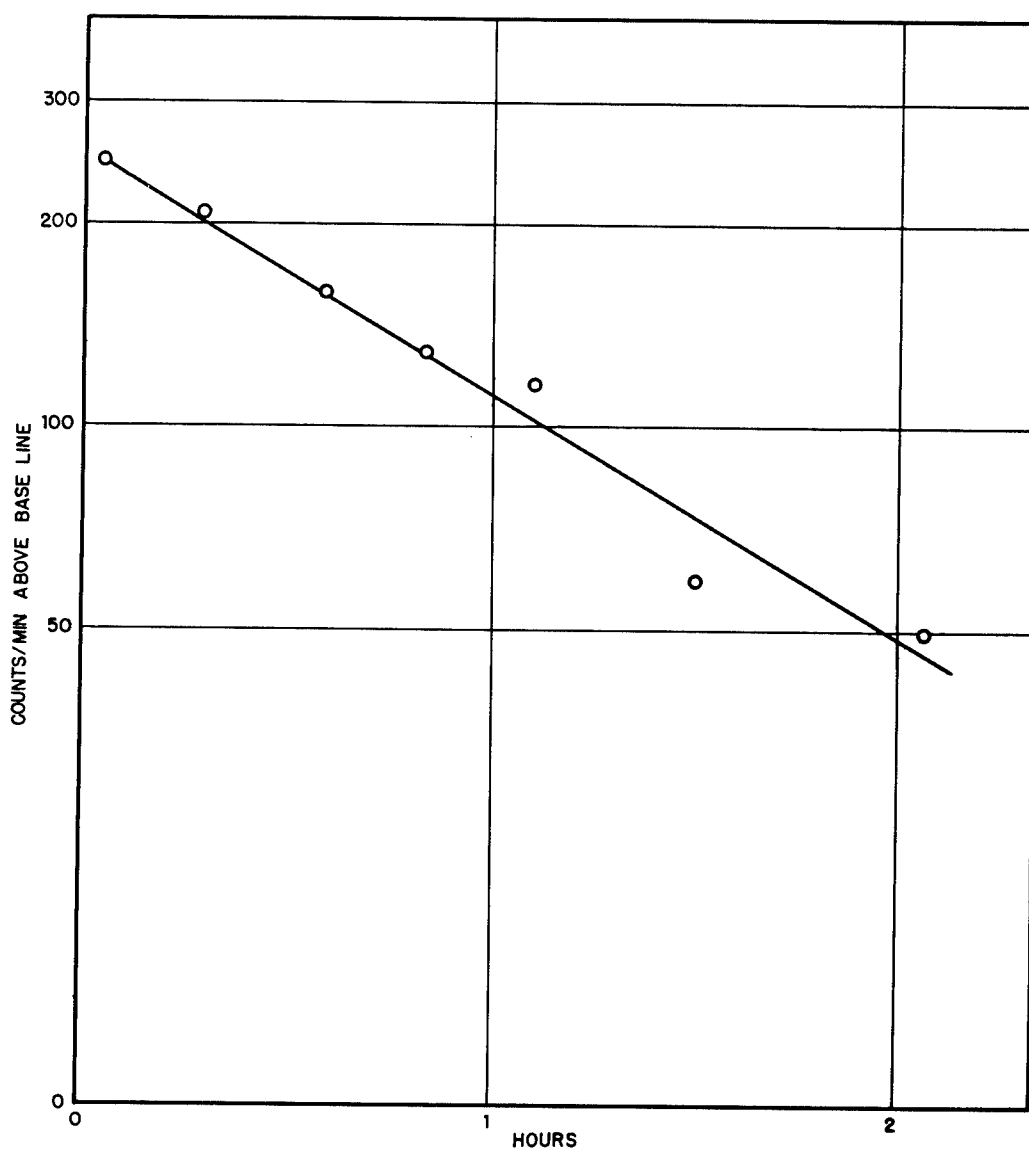


Figure 5. Semi-logarithmic plot taken from the data of Figure 4 showing the exponential character of the initial portion of the curve.

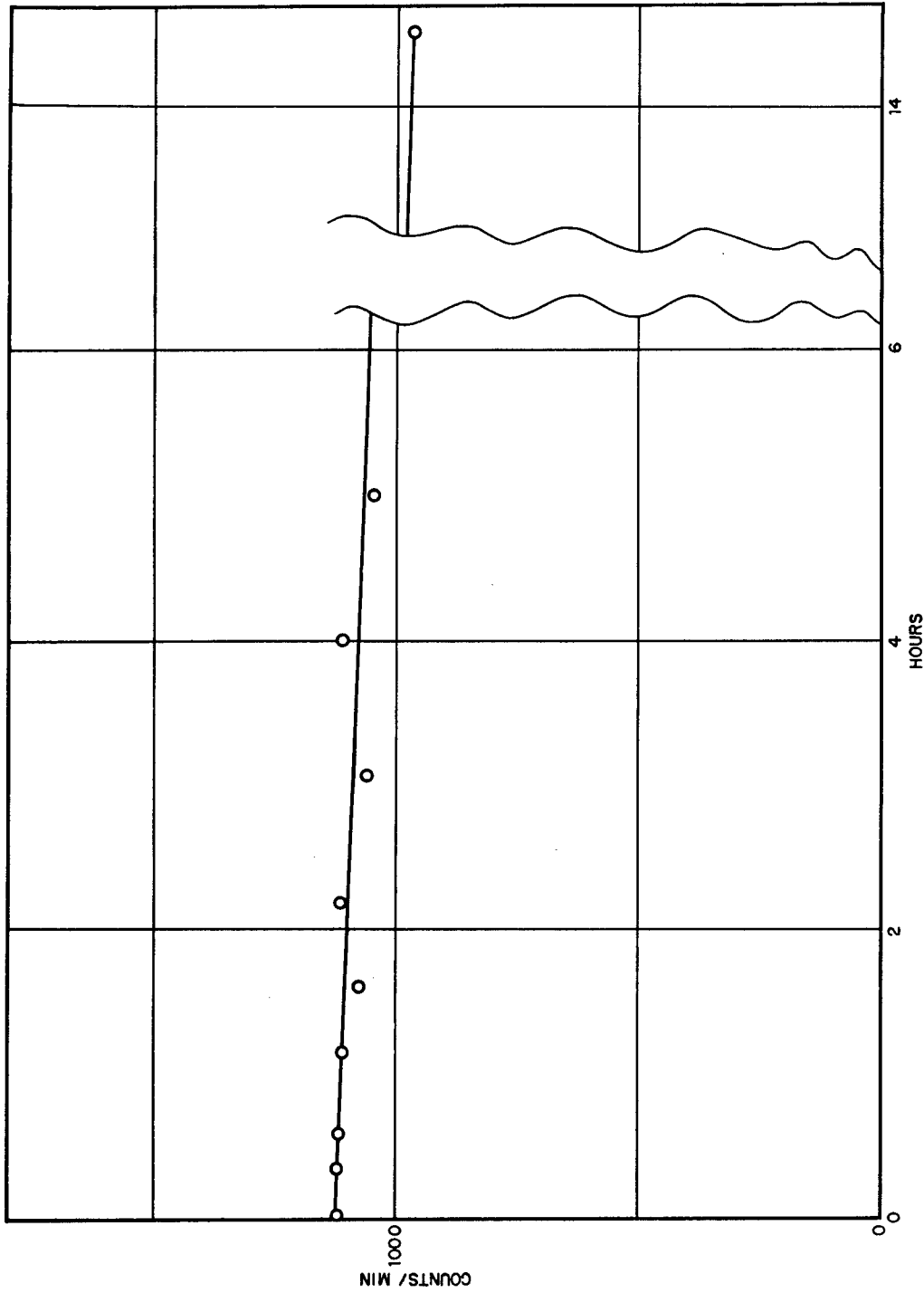


Figure 6. Decrease of plasma activity of blood from which the fraction containing white cells and platelets has been removed.

It is remotely possible that part of the initial rapid exchange occurred in some fraction of the red cells which was removed with the top layers of cells during the differential centrifugation procedure. We hope soon to have further information on this point. At the same time we will investigate the contribution of the platelets to the exchange process.

We can make a rough estimate of the amount of easily exchangeable cellular potassium involved in the typical case shown in Figure 4. Here, after the rapid exchange was complete, about 25% of the radioactivity had evidently left the plasma and mixed uniformly with the exchangeable potassium of the white cell-platelet fraction. The ratio of total radioactivity then present in the two fractions was thus 1 to 3. If we assume that mixing was then complete, this ratio is the same as that of easily exchangeable cellular potassium to total plasma potassium. If we reasonably assume the total volume of white cells and platelets was about 2% of that of the plasma then the ratio of the concentration of exchangeable potassium in these cells to that in the plasma was about 17 to 1. This is not unreasonable since many cells are known to have a high content of potassium relative to sodium.

Our findings indicate one point very clearly. If studies of erythrocyte exchange are to be put on a precise basis, it will be necessary in future experimental work to remove all cellular elements not belonging to the erythrocyte series or to take the effect of this extraneous population into account in some other manner.

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